



Vinogradov, S. N., Hoogewijs, D., Bailly, X., Arredondo-Peter, R., Gough, J. J. T., Dewilde, S., Moens, L., & Vanfleteren, J. R. (2006). A phylogenomic profile of globins. *BMC Evolutionary Biology*, 6, [31]. <https://doi.org/10.1186/1471-2148-6-31>

Publisher's PDF, also known as Version of record

License (if available):
CC BY

Link to published version (if available):
[10.1186/1471-2148-6-31](https://doi.org/10.1186/1471-2148-6-31)

[Link to publication record in Explore Bristol Research](#)
PDF-document

This is the final published version of the article (version of record). It first appeared online via BioMed Central at <http://bmcevolbiol.biomedcentral.com/articles/10.1186/1471-2148-6-31>. Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available: <http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

Research article

Open Access

A phylogenomic profile of globins

Serge N Vinogradov^{*1}, David Hoogewijs², Xavier Bailly³, Raúl Arredondo-Peter⁴, Julian Gough⁵, Sylvia Dewilde⁶, Luc Moens⁶ and Jacques R Vanfleteren²

Address: ¹Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI 48201, USA, ²Department of Biology, Ghent University, B-9000 Ghent, Belgium, ³Station Biologique de Roscoff, 29680 Roscoff, France, ⁴Laboratorio de Biofísica y Biología Molecular, Facultad de Ciencias, Universidad Autónoma del Estado de Morelos, 62210 Cuernavaca, Morelos, México, ⁵RIKEN Genomic Sciences Centre, Yokohama 230-0045, Japan and ⁶Department of Biomedical Sciences, University of Antwerp, 2610 Antwerp, Belgium

Email: Serge N Vinogradov^{*} - svinogra@med.wayne.edu; David Hoogewijs - david.hoogewijs@UGent.be; Xavier Bailly - bailly@sb-roscoff.fr; Raúl Arredondo-Peter - normanac@terra.com.mx; Julian Gough - gough@gsc.riken.jp; Sylvia Dewilde - sylvia.dewilde@ua.ac.be; Luc Moens - luc.moens@ua.ac.be; Jacques R Vanfleteren - jacques.vanfleteren@UGent.be

^{*} Corresponding author

Published: 07 April 2006

Received: 15 December 2005

BMC Evolutionary Biology 2006, 6:31 doi:10.1186/1471-2148-6-31

Accepted: 07 April 2006

This article is available from: <http://www.biomedcentral.com/1471-2148/6/31>

© 2006 Vinogradov et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: Globins occur in all three kingdoms of life: they can be classified into single-domain globins and chimeric globins. The latter comprise the flavohemoglobins with a C-terminal FAD-binding domain and the gene-regulating globin coupled sensors, with variable C-terminal domains. The single-domain globins encompass sequences related to chimeric globins and «truncated» hemoglobins with a 2-over-2 instead of the canonical 3-over-3 α -helical fold.

Results: A census of globins in 26 archaeal, 245 bacterial and 49 eukaryote genomes was carried out. Only ~25% of archaea have globins, including globin coupled sensors, related single domain globins and 2-over-2 globins. From one to seven globins per genome were found in ~65% of the bacterial genomes: the presence and number of globins are positively correlated with genome size. Globins appear to be mostly absent in Bacteroidetes/Chlorobi, Chlamydia, Lactobacillales, Mollicutes, Rickettsiales, Pastorellales and Spirochaetes. Single domain globins occur in metazoans and flavohemoglobins are found in fungi, diplomonads and mycetozoans. Although red algae have single domain globins, including 2-over-2 globins, the green algae and ciliates have only 2-over-2 globins. Plants have symbiotic and nonsymbiotic single domain hemoglobins and 2-over-2 hemoglobins. Over 90% of eukaryotes have globins: the nematode *Caenorhabditis* has the most putative globins, ~33. No globins occur in the parasitic, unicellular eukaryotes such as *Encephalitozoon*, *Entamoeba*, *Plasmodium* and *Trypanosoma*.

Conclusion: Although Bacteria have all three types of globins, Archaea do not have flavohemoglobins and Eukaryotes lack globin coupled sensors. Since the hemoglobins in organisms other than animals are enzymes or sensors, it is likely that the evolution of an oxygen transport function accompanied the emergence of multicellular animals.

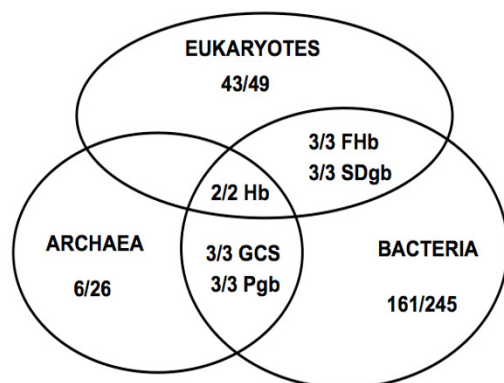


Figure 1

A Venn diagram of the distribution in the three kingdoms of life, of the three globin lineages: 3/3 FHbs/SDgbs, 3/3 GCSs/Pgbs and 2/2 Hbs. The number of genomes that have globins is shown as a fraction of the total number of genomes.

Background

The number of globin families has grown markedly since the 1970's, when they were limited to the α - and β -globins and Mbs, mostly of vertebrates, and the SHbs (legHbs) of legume plants [1-3]. The ensuing years brought to light several new globins: SHbs in plants other than legumes, NsHbs in a wide variety of plants [4-6], and FHbs, chimeric proteins (~400aa) comprising an N-terminal globin domain and a C-terminal FAD- and NAD-binding domain, related to the ferredoxin-NADP⁺ reductases, found in *E. coli* [7] and in yeasts [8,9]. Concurrently, "truncated" globins, sequences shorter than normal (<130aa), were discovered in protozoa [10], cyanobacteria [11-13], a nemertean [14], and bacteria [15,16]. Globins longer than normal (>160aa), similar to the "truncated" Hbs, were observed in a green alga [17,18] and in plants [19]. The crystal structures of several "truncated" Hbs showed them to have a novel 2-over-2 α -helical fold instead of the canonical 3-over-3 α -helical fold, with an abbreviated A helix, a decreased CE interhelical region and most of the F helix occurring as a loop [20-22]. Consequently, we advocate using 2/2 Hb instead of "truncated", to indicate the distinctive secondary structure of this group of globins and to bring order to the chaotic terminology in the existing databases, where "truncated", "cyanobacterial", "protozoan" and "2-over-2" are all in current use.

The utilization of molecular biological techniques allowed the detection of globins in organisms where their presence was unsuspected, such as the nematode *Caenorhabditis elegans* and in other nematodes [23-30], the

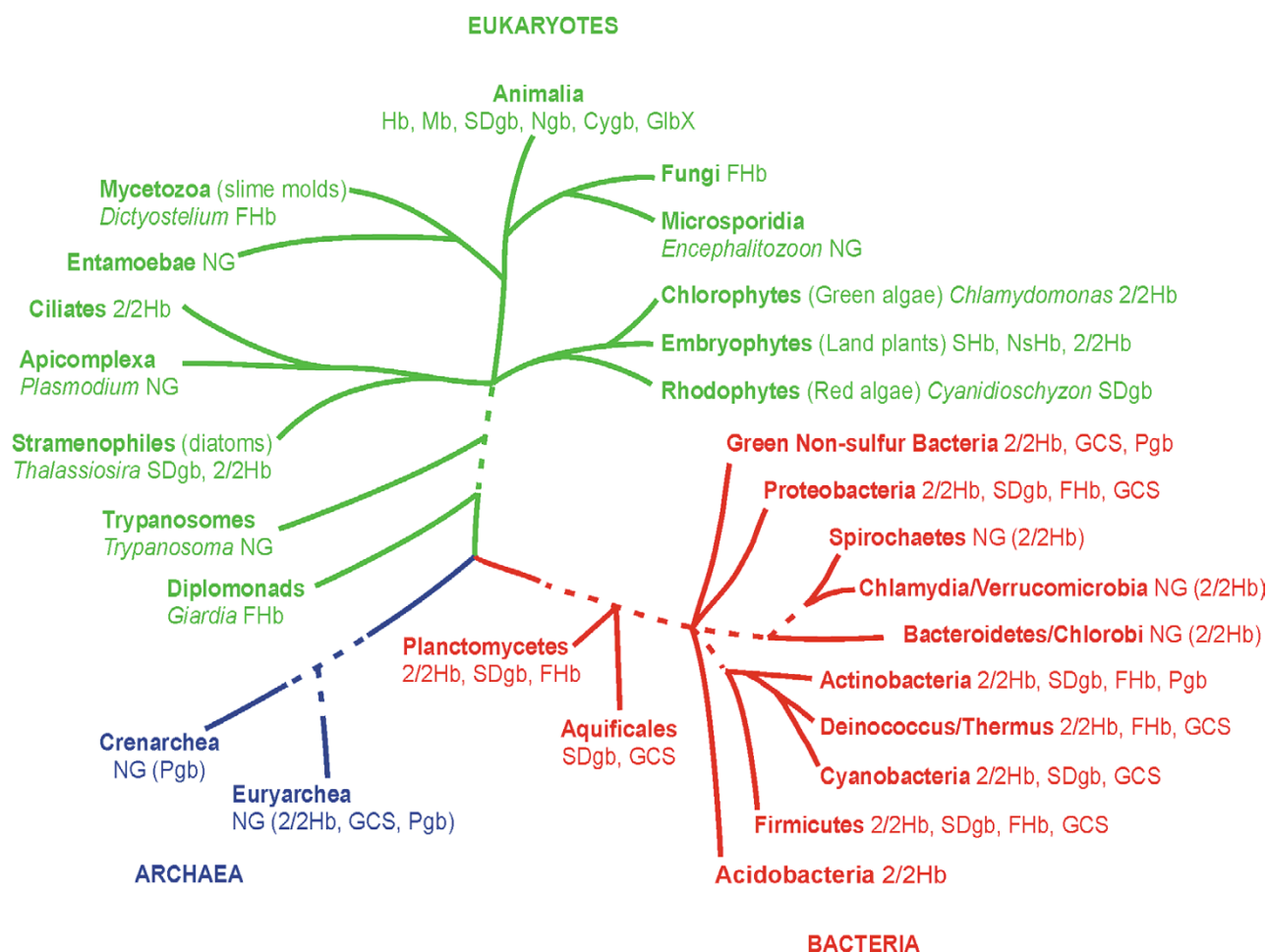
dipteran *Drosophila melanogaster* [31] and the urochordate *Ciona intestinalis* [32]. The recent, rapid accumulation of genomic information has resulted in a substantial increase in newly recognized globins. This list includes the Ngbs [33,34] and Cygbs [35-37], which are believed to occur in all vertebrates from humans to birds and fish [38], GbE, the eye-specific globin in the domestic chicken *Gallus gallus*, related to Cygb [39], and GbX, a new and fifth type of globin gene in fish and amphibians, thought to have been lost in the higher vertebrates [40]. FHbs were found in a variety of bacterial groups [16,41-43] together with SDgbs, which align with the FHb globin domains [42,43]. Furthermore, globin coupled sensor proteins, chimeric two-domain gene regulators (~300 to >700aa) comprising an N-terminal globin domain, were discovered in an archaean and several bacterial groups [44-47]. Lastly, "protoglobins" (~195aa) related to the former, were found in archaea and in several bacteria [48] and proposed to represent the "ancestral" globin.

Results

Overview

The 245 bacterial, 26 archaeal and 49 eukaryote genomes, and the identified known and putative globins, are listed in Supplemental Data Tables 1-6 [see Additional File 1]. An alignment of the sequences is provided in Supplemental Data Fig. 1 [see Additional File 2]. All the globins identified in our survey can be assigned to two general classes: single chain globins and globin domains within chimeric proteins. The former group comprises the vertebrate α - and β -globins, Mbs, Ngbs and Cygbs, the metazoan intra- and extracellular Hbs (including multi-domain and/or multisubunit Hbs), the plant SHbs and NsHbs, the ~195aa Pgbs and the 2/2 Hbs. The two groups of chimeric proteins, which generally have an N-terminal globin domain, are the ~400aa FHbs with an NAD and FAD-binding C-terminal domain, and the GCSs with highly variable (~50 to >700a) C-terminal domains. Thus, all globins belong to one of three globin lineages: the 3/3 FHbs and SDgbs (eukaryote and bacterial), the 3/3 GCSs and Pgbs and the 2/2 SDgbs [49]. Fig. 1 provides the number of genomes that have globins and illustrates a key result: only one of the three globin lineages, the 2/2 Hbs, are represented in all three kingdoms of life. Fig. 2 shows the approximate phylogenetic relationships between the main groups of the three kingdoms of life based on Baldauf et al. [50] and depicts the phylogenomic profile of globins.

It should be emphasized, that the presence of putative globins inferred using SUPERFAMILY and blastp and psiblast searches of the GenBank database, does not require having a completed genome. On the other hand, the absence of a globin is certain only when a complete genome is searched and no sequence is found.

**Figure 2**

A diagrammatic representation of the three kingdoms of life and the phylogenetic relationships of their major groups based on Baldauf et al. [50], and depicting the distribution of globins.

Archaea

Table 2 in the Supporting Data [see Additional File 1] lists the archaeal genomes, their sizes and the presence of globins. SUPERFAMILY identified putative globins in four Archaea: *Halobacterium*, *Methanobacterium*, *Methanosarcina* and *Sulfolobus*. In addition, another globin was identified in the SWISSPROT/TrEMBL database (tr|Q96ZL6). Examination of these sequences showed that they are not likely to be globins due to lack of size (< 100aa) and/or the absence of a suitable F8His. However, Hou et al.

[44,45,48] have recently discovered GCSs and Pgbs in a couple of archaea, and we found a 2/2 Hb1 in *Haloarcula marismortui* [49]. This survey has uncovered a 2/2 Hb1 in still another euryarcheote halophile *Haloferax volcanii*. Thus, only five of the 20 Euryarcheote genomes have globins: *Haloarcula marismortui* (2/2 Hb1, GCS), *Halobacterium salinarum* (GCS), *Haloferax volcanii* (2/2 Hb1, GCS), *Methanosarcina acetivorans* and *M. barkeri* (Pgbs). Of the 5 Crenarcheote genomes, only *Aeropyrum pernix*, has a globin (Pgb) and the only Nanoarcheote genome has no globins.

Table 1: Number of bacterial genomes with one or more classes of 2-over-2 Hbs

Class 1	Class 2	Class 3	Class 1+2	Class 1+3	Class 2+3
17	47	18	14	1	18

The paucity of globins in Archaea is one of the surprising results of our survey. Except for *Aeropyrum pernix*, the archaeal genomes comprising globins are, coincidentally, among the largest known archaeal genomes. It is interesting to note that none of the GCSs or Pgbs have been identified by the hidden Markov chain model used by SUPERFAM, although they align readily with the globin fold, based on the recent crystal structure of *Bacillus subtilis* GCS [51]. Most of the archaeal genomes sequenced to date have been those of extremophilic organisms with small genomes. It is now known that there are many more non-thermophilic terrestrial and marine archaea than hitherto suspected [52]. It remains to be seen whether additional archaeal genomes will reverse the situation.

Bacteria

Table 3 in Supporting Data [see Additional File 1] lists the completed and unfinished bacterial genomes used in this study, their sizes, the type and number of globins present, 2/2 Hbs (classes 1, 2 and 3), FHbs (SDgbs) and GCSs (Pgbs), and the oxygen requirement and habitat of the organism. A diagrammatic representation of the phylogenetic relationships between the main bacterial groups based on Baldauf *et al.* [50], shown in Fig. 3, summarizes the globin distribution among the bacteria. The one representative of the Acidobacteria has globins, and only 4 of the 26 genomes representing the Actinomycetes have none: *Bifidobacterium*, *Propionibacterium*, *Symbiobacterium* and *Tropheryma*. The 3 Aquificales/Thermotogales have globins except *Thermotoga*, and the 5 Bacteroidetes/Chlorobi (except *Cytophaga*) lack globins, as well as the 6 Chlamydiae/Verrumicrobium genomes, except *Parachlamydia* and *Verrumicrobium*. Of the 3 Chloroflexi genomes, *Dehalococcoides*, has no globins. The Cyanobacteria are represented by 12 genomes, of which 5 lack globins: *Anabaena*, *Crocospaera*, *Prochlorococcus*, *Synechococcus elongatus* and *Trichodesmium*. Both *Deinococcus* and *Thermus* have globins. Among the 49 Firmicutes, apart from the Lactobacillales and the Mollicutes, the 17 Bacilli have globins (except *Listeria*), as well as 4 of the 7 Clostridia. Although *Fusobacterium* lacks globins, the lone Nitrospirae and the two Planctomycetes have globins. The majority of the 35 genomes of Alphaproteobacteria have

globins: all the Caulobacterales, Rhizobiales (except *Bartonella*), Rhodobacterales, Rhodospirillales and Sphingomonadales, but only one of the 10 Rickettsiales (*Pelagibacter ubique*). All 24 genomes representing the Betaproteobacteria have globins, except 2 of the 4 Neisseriaceae. The 50 Gammaproteobacteria genomes represent 10 groups, of which only 3 of the 10 Enterobacteriales (*Buchnera*, *Blochmannia* and *Wigglesworthia*), one of two Legionellales, the 5 Pasteurellales, the two *Psychrobacter* of the 10 Pseudomonadales, and the one Thiotrichales (*Francisella*) are devoid of globins. The 10 genomes of Deltaproteobacteria have globins, except for *Desulfovibrio*. Two of the 8 genomes representing the Epsilonproteobacteria have no globins: *Helicobacter pylori* and *Wolinella*; however, *Helicobacter hepaticus* has a globin. The lone unassigned proteobacterium *Magnetococcus* has a GCS. Of the 128 genomes representing the Proteobacteria, 99 have globins (~75%).

Alignment of bacterial 2/2 Hb sequences indicates that they can be divided into three separate classes [16,53]. The length of the 2/2 Hbs varies from 118aa for the cyanobacterium *Nostoc* (ZP_00112318) to 260aa in *Streptomyces avermitilis* (NP_824492), with the majority (>60) being <140aa. Examination of coexistence of the three classes of 2/2 Hbs (Table 1) shows an interesting trend: although only the Alphaproteobacterium *Hyphomonas neptunium* has both 1 and 3 and two have all three classes, 14 genomes have classes 1 and 2 and 18 have classes 2 and 3. These results suggest that the class 1 and 3 2/2 Hbs are derived from the class 2 2/2 Hbs, as pointed out very recently by Vuletich and Lecomte [53].

The finding of an FHb in *E. coli* [7] led to the discovery of some 30 bacterial FHbs [16,42,43,54]; currently, the number is over 70 (Supplementary Data Table 3). R. Poole and his group [43] and Frey and Kallio [42] were the first to point out the similarity between the FHb globin domains, the first bacterial Hb to be sequenced, that of *Vitreoscilla stercoraria* [55], and several other SD bacterial globins. We now have a total of 25 SDgbs in 22 genomes. The crystal structures of the *P. aeruginosa* (PDB: [1tu9](#)) [56] and *Vitreoscilla stercoraria* (PDB: [2vhh](#)) [57],

Table 2: Coexistence of FHbs with SDgbs in bacterial genomes.

FHb only	SDgb only	FHb+SDgb
65	18	6

Table 3: Coexistence of FHbs/SDgbs with 2-over-2 Hbs in bacterial genomes.

FHb or SDgb only	With class 1	With class 2	With class 3	With class 1+2	With class 1+3	With class 2+3
26	4	29	7	2	0	13

show them to be very similar to the globin domains of the FHbs from *E. coli* (PDB: [1gvh](#)) [58] and *Ralstonia* (*Alcaligenes*) *eutropha* (PDB: [1cqx](#)) [59]. It is appropriate to note here that *Ralstonia* has undergone two name alterations recently, to *Wautersia eutropha* and now to *Cupriavidus necator* [60]. Although 22 genomes have SDgbs, three of them have two different globins: *Bradyrhizobium*, *Rhodopseudomonas* and *Novosphingobium*. Table 2 shows that FHbs and SDgbs coexist in only 6 genomes: *Chromobacte-*

rium, *Photobacterium*, *Pseudomonas aeruginosa*, *Rhodopirellula*, *Thermobifida* and *Vibrio parahaemolyticus*. Table 3 shows the statistics of occurrence of 2/2 Hbs with the FHbs/SDgbs; the latter tend to occur with class 2 and with both class 2 and 3 2/2 Hbs.

M. Alam and his group have identified 27 GCSs [46,47] and 3 Pgbs from bacteria – *Chloroflexus aurantiacus* ZP_00359040 (227aa), *Thermobifida fusca* ZP_00293478

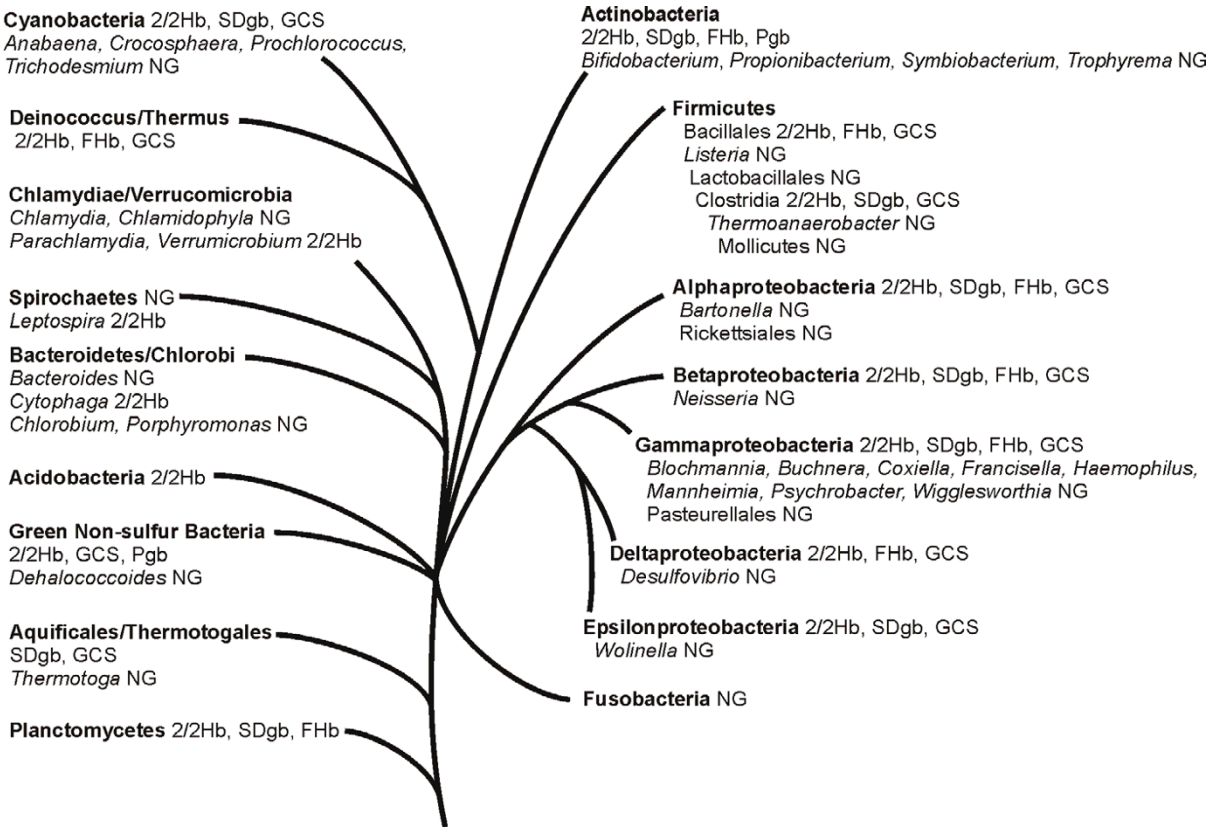


Figure 3
A diagrammatic representation of the phylogenetic relationships between the major bacterial groups based on Baldauf et al.[50], and showing the distribution of globins.

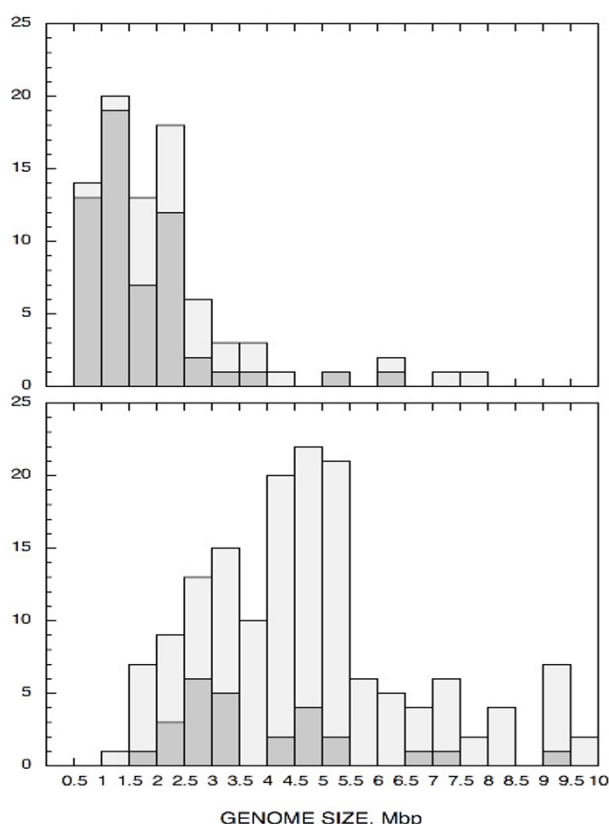


Figure 4
The statistics of bacterial genome size distribution: lacking globins (upper panel) and containing globins (lower panel). The genomes that are host-associated (Table 3 in Supplementary Material) are represented by stippled bars.

(197aa) and *Thermosynechococcus elongatus* NP_682779 (194aa) [48]. Blastp searches found an additional two dozen GCSs, and Pgbs in *Thermus thermophilus* YP_005074 (203aa) and the actinobacterium *Rubrobacter xylanophilus* ZP_00200180 (196aa) [49,61]. The recent crystal structure of *B. subtilis* GCS shows it to have a 3/3 fold [62].

The genomes with the largest number of globins, 5 to 7, are all from the Alpha- and Betaproteobacteria; they do include however, redundant sequences. The following have all three lineages of globins: *Burkholderia fungorum* (9.67 Mbp), *Chromobacterium violaceum* (4.75 Mbp), *Novosphingobium aromaticivorans* (4.21 Mbp) and *Silicibacter* sp.TM1040 (4.14 Mbp), each with 5 globins, the three *Bordetella* species (4.09–5.34 Mbp), *Exiguobacterium* sp.255–15 (2.89 Mbp), *Shewanella baltica* (5.01 Mbp), *Sinorhizobium meliloti* and *Thermobifida fusca* (3.64 Mbp), each with 4 globins, and *Azotobacter vinelandii* (5.42 Mbp), all the *Bacillus* species (except *B. licheniformis* and *B. stearothermophilus*) (4.20–5.50 Mbp) and *Geobacillus kaus-*

tophilus (3.59 Mbp), each with 3 globins. At the other end of the scale, the smallest genome (1.31 Mbp) to have a globin, a 2/2 Hb1, is that of the marine Alphaproteobacterium *Pelagibacter ubiques* (Rickettsiales), followed by *Aquifex aeolicus* (1.59 Mbp), and the Epsilonbacterium *Campylobacter jejuni* (1.64 Mbp), with one (SDgb) and two (2/2Hb and SDgb) globins, respectively.

The bacterial divisions represented by 10 or more genomes, rank in the following order of having globins: Betaproteobacteria – 92% (22/24) > Actinobacteria – 85% (22/26) > Gammaproteobacteria – 76% (38/50) > Alphaproteobacteria – 69% (24/35) > Cyanobacteria – 58% (7/12) > Firmicutes – 49% (19/39). Overall, 161 of the 245 (~65%) bacterial genomes have globins and the number of globins varies from 1 to 7.

Fig. 4 depicts the size distribution of bacterial genomes lacking globins (upper panel) and comprising globins (lower panel); the stippled columns represent the genomes associated with a host (given in Supplemental Data Table 3 [see Additional File 1]). Although the two distributions overlap, it is evident that presence of globins is correlated with genome size and that the majority of genomes lacking globins are host associated. Table 4 shows the mean genome size for completed genomes lacking globin and with one or more globins. The mean size of the genomes shows a positive correlation with the number of globins present. About 80% (65/82) of genomes <2.5 Mbp lack globins (mean 2.1 Mbp). Very small genomes are characteristic of bacteria with lifestyles characterized by continuous association with a host [63]. The majority of the bacteria whose genomes have been sequenced, are human, animal and plant infectious agents. Thus, it is likely that the estimate of approximately two thirds of all bacteria having globins obtained in the present study, is on the low side.

Eukaryotes

The globins identified in eukaryote genomes are listed in Supplemental Data Tables 4–6 [see Additional File 1]. The globins listed for the vertebrate genomes, *Danio (Brachydanio) rerio*, *Fugu (Takifugu) rubripes*, *Gallus gallus*, *Homo sapiens*, *Mus musculus*, *Rattus norvegicus* and *Xenopus tropicalis*, include the familiar α - and β -globins and Mb, as well as the recently discovered Ngbs [33,34] and Cygbs [35–37]. A recent addition to human α -globins is μ -globin (gi|51510893|NP_001003938, 141aa) which appears to be very similar to the avian α -D globin [64]. In addition, an eye-specific globin, GbE, was found in *Gallus gallus* [39] and a new globin, GbX, was discovered in the fish, bird and amphibian genomes [40]. Curiously, no Mb-like sequence was found in either *Xenopus tropicalis* or *X. laevis*. The zebrafish *Danio rerio* has 6 embryonic globins [65], three α and three β - only 5 are listed in the GenBank. The

Table 4: Number of globins found in bacterial genomes and the corresponding mean genome sizes.

No. of globins	No of completed genomes	Mean genome size, Mbp
0	75	1.9 ± 1.1
1	40	3.9 ± 1.5
2	19	4.2 ± 1.2
3	22	4.7 ± 1.2
4	11	6.5 ± 1.9

pufferfish *Fugu rubripes*, appears to have four α -globin chains [66]; only three of them are listed in the GenBank.

The four globins found in the genome of the urochordate *Ciona intestinalis* (sea squirt), have been identified earlier [32]. This important finding corrects the erroneous claim of total absence of globins in the report of the genome [67].

At least one putative globin (gi|72169631|XP_795670, 175aa) was found in the recently completed genome assembly of the echinoderm *Strongylocentrotus purpuratus* (sea urchin). Used as query in blastp searches it hits the GlbXs and Cygbs of fish, and other vertebrate Cygbs, before recognizing the coelomic globin (gi|729576;P80018) of a fellow echinoderm, the holothurian *Caudina arenicola*.

There appear to be 4 putative globins in *Anopheles gambiae*: gi|55246163|EAA03862.3 (150aa), gi|31201271|XP_309583 (192aa), gi|31198253|XP_308074 (215aa) and gi|57914327|XP_555006 (182aa). Their alignment shows that the first three are closely related, sharing essentially identical globin domains: gi|55246163 has an 18aa deletion in the BC helix and the CD corner, while gi|31198253 lacks part of the G and all of the H helices. Thus, it is likely that gi|31201271 and gi|57914327 are the only stable globins. The C-terminal 115aa globin domain of gi|31198253 is reminiscent of the Mb gene sequence from the antarctic icefish *Champscephalus esox* (gi|24266940|AAN52371, 103aa) that does not appear to be expressed [68]. It remains to be determined whether the N-terminal domain of gi|31198253 stabilizes it enough to be expressed.

At least 33 putative globins and globin domains were identified in *Caenorhabditis elegans*, together with their orthologs in *C. briggsae* [30]: they are listed in Supplemental Data Table 5 [see Additional File 1]; their alignment is provided in Supplemental Data Fig. 2 [see Additional File 2]. Several *C. elegans*/*C. briggsae* orthologs have unusually long interhelical inserts between the G and H helices:

40aa in CE01012/CBP03870) (216aa), 21aa in CE01528 (CBP00622) (266aa), 22aa in CE34964/CBP23619 (196aa) and 21aa in CE34658/CBP07299 (230aa). Although three pairs, CE17437/CBP02580, CE04582/CBP03989 and CE35828/CBP14482, have N-terminal globin domains, several have C-terminal domains: CE03523/CBP15914, CE04843/CBP01576, CE05316/CBP02907, CE12774/CBP21478), CE36044/CBP02078), CE29586/CBP02293, CE30683/CBP02390 and CE31132/CBP15597. The N-terminal portion of the latter is identified as a G protein-coupled receptor-like domain with 7 putative transmembrane helices (identified using TMHMM Server V.2.0). The nonglobin domains in the remaining proteins could not be identified via blastp searches.

Although globin genes were thought to be absent in the genome of *Drosophila melanogaster* [69], the presence of at least one (CG9734) has been demonstrated unequivocally by Burmester and Hankeln [31,70]. There appear to be two more putative globins, CG15180 (209aa) and CG14675 (195aa); both provide acceptable alignments. Three putative globins are also found in *D. pseudoobscura*, EAL28410 (152aa), EAL28093 (641aa, 40–150) and EAL28094 (130aa) [71], and appear to be orthologs of *D. melanogaster* CG9734, CG15180 and CG14675, respectively. Although the *D. pseudoobscura* EAL28093 and EAL28094 are scored by FUGUE as certainly globins ($Z \sim 10$), the former lacks an appropriate F8 His residue and the latter appears to be missing the A helix. Psiblast searches show that the ortholog pairs CG15180/EAL28093 and CG14675/EAL28094 recognize each other but not the CG9734/EAL28410 pair, and also recognize the *Anopheles* globins (ENSANG19788, 22287 and 26474), together with distant recognition of echinoderm globins and vertebrate β -globins. The CG9734/EAL28410 pair hits the other known insect globins (*Gasterophilus*, *Chironomus* species, *Kiefferulus*, *Tokunagayusurika*), the vertebrate Cygbs and the arthropod multidomain Hbs.

Three of the four *Arabidopsis thaliana* sequences, GLBs 1–3 have been identified earlier [19,72]: GLB1 and GLB2 are NsHbs and GLB3 is a 2/2Hb. A 693aa protein

(gi|7486404|T04457) was found to have an N-terminal 2/2Hb domain, with the first 138aa (up to position H13), identical to GLB3. Although the ~500aa C-terminal portion does not correspond to any known protein domain(s), a blastp search using it as query found an identical sequence in the N-terminal portion of the 2154aa protein TEBICHI (gi|62241195|BAD93700). Both proteins share the same region on *Arabidopsis* chromosome 4 and TEBICHI has a helicase domain at 490–890aa and a polymerase domain at 1700–2150aa; furthermore, this N-terminal portion is missing in animal homologues of TEB protein, MUS308/POLQ (Dr. Soichi Inagaki, personal communication).

The genome of *Oryza sativa* contains the four NsHbs identified by Arredondo-Peter et al. [73,74], a 172aa 2/2Hb (gi|50725383|BAD32857) and a 145aa globin (gi|50932383|XP_475719). A blastp search identified the latter as another NsHbs.

Several putative globins occur in the genome of the green alga *Chlamydomonas reinhardtii*: 160981 (C_240146) (136aa), 160982 (C_240147) (147aa), 157690 (C_1780015) (231aa), and two larger sequences, 168934 (C_60169) (476aa) with one N-terminal globin-like domain, and 153190 (C_100138) (837aa) with two consecutive N-terminal globin domains. Two globins were found in the genome of the diatom *Phaeodactylum tricornutum*: PTMM3909 and 05212. Two globins occur in the genome of another diatom *Thalassiosira pseudonana*, Scaffold_137 (158aa) and Scaffold_18 (160aa), and a single 185aa globin, CMR319C, in the genome of the red alga *Cyanidioschyzon merolae*. Blastp searches identified all the *C. reinhardtii* globins, the *Phaeodactylum* PTMM 05212 and the *Thalassiosira* Scaffold_18 as 2/2 Hbs, and the *Cyanidioschyzon* CMR319C, *Phaeodactylum* PTMM 3909 and *Thalassiosira* Scaffold_137 as SDgbs, closely related to the bacterial and fungal FHbs and the bacterial SDgbs.

The globins present in the fungi are listed in Supplemental Data Table 6 [see Additional File 1]. All the recently completed fungal genomes, which belong overwhelmingly to the Ascomycota (17 of 19), have FHbs; 14 have two to four FHbs, probably due to genome duplication [75]. In addition to *Saccharomyces cerevisiae* NP_014165 (426aa, 154–302) which was known to have a central globin domain [76], we found an additional 12 FHbs to have globin domains within the central portion of their sequences. All belong to the Saccharomycotina: *Candida albicans* EAK92722 (563aa, 298–463), *Candida glabrata* XP_448033 (432aa, 124–267), *Eremothecium (Ashbya) gossypii* NP_982746 (436aa, 184–339), *Kluyveromyces lactis* XP_453939 (430aa, 181–323), *Kluyveromyces waltii* Kwal_22190 (421aa, 171–314) Kwal_4395 (460aa, 202–352) and Kwal_24852 (543, 173–315), *Saccharomyces*

bayanus ORFP:20532(424aa, 171–303), *Saccharomyces mikatae* ORFP:18051 (410aa, 135–290), *Saccharomyces paradoxus* ORFP:18484 (426aa, 155–306) and *Yarrowia lipolytica* XP_502881 (463aa, 186–325) and XP_499869 (471aa, 194–333). Although the globin domains align well with the other FHbs, the N- and C-terminal portions do not recognize the C-terminal moieties of the other fungal and bacterial FHbs or any other known protein, as found earlier for the *S. cerevisiae* FHb [76]. Surprisingly, the percent identities between the CDFHbs and the fungal and bacterial FHb globin domains is about 40% and 30%, respectively.

Among the lower eukaryotes, the diplomonad *Giardia lamblia* has one FHb [77] and the mycetozoan *Dictyostelium discoideum* has two [78]. The *Giardia* sequence has a 21aa insert between helices E and F.

No globin genes appear to exist in the genomes of the Apicomplexans *Entamoeba histolytica* and *Plasmodium falciparum*, as well as the Microsporidian *Encephalitozoon cuniculi* and the Euglenozoan *Trypanosoma brucei*. Although a *Plasmodium bergeri* globin-like protein of 228aa (Q86QI8) is listed in the GenBank, it is probably an artefact, since when used as a query in blastp searches, it hits only vertebrate β - and α -globins.

Compared to Archaea and Bacteria, the fraction of eukaryote genomes with globins is much higher, over 90%. Although all vertebrates have Hb and Mb and probably Ngb and Cygb as well [38], the Antarctic icefish belonging to the family Channichthyidae do not express Hb [79]. Furthermore, at least 6 of the 16 icefish species also do not express Mb [80]. The lack of Hb is due apparently to deletion of the β -globin gene [81], which occurred during the last 10 to 16 Myr, the approximate date of divergence of the Notothenioid lineage including the Channichthyidae [82]. In contrast, the lack of Mb appears to be due to errors in Mb gene transcription [68]. It is not known whether the icefish have Ngbs and/or Cygbs. In the remaining two chordate phyla, the urochordate *Ciona* has 4 globins (see above), which when used as queries in psiblast searches, recognize the vertebrate globins and the eukaryote and bacterial FHbs and SDgbs. The report of a Hb in the notochord of the cephalochordate amphioxus (*Branchiostoma californiense*) [83], suggests that globins are present in all chordates. Among the remaining deuterostome phyla, intracellular Hbs have been reported in two of the 5 classes of echinoderms [84,85], and we find a putative globin in the recent assembly of the genome from the sea urchin *Strongylocentrotus purpuratus*, which belongs to another class. No globins have been reported so far in hemichordates.

Among the remaining metazoans, direct visual observation of Hbs is highly episodic among the metazoans, and varies from total absence of Hb in porifera and cnidaria, to frequent presence in some nematode and platyhelminth groups [3]. In the largest metazoan group, the insects, of which about 900,000 species have been described, and which outnumber the combined total of all other animal species, with estimates ranging from 2×10^6 to 100×10^6 [86], globins are so far known to be present only in Dipterans, such as *Gasterophilus*, *Drosophila* and *Chironomus*. It is evident that much work needs to be done before we have any clear idea of globin distribution in metazoans other than deuterostomes.

Among the lower eukaryotes, our census suggests that globins could be ubiquitous in fungi (FHbs), mycetozoa (FHbs), diplomonads (FHbs), ciliates (2/2 Hbs), stramenopiles (SDgb, 2/2Hb), rhodophytes (SDgb), chlorophytes (2/2Hb) and plants (SHbs, NsHbs, 2/2 Hbs). In contrast, the genomes of pathogenic microsporidians, entamoebae, apicomplexans and trypanosomes are devoid of globins. In view of a very large potential diversity of small, bacterial-sized eukaryotes revealed by recent, culture-independent surveys [87,88], much remains to be done.

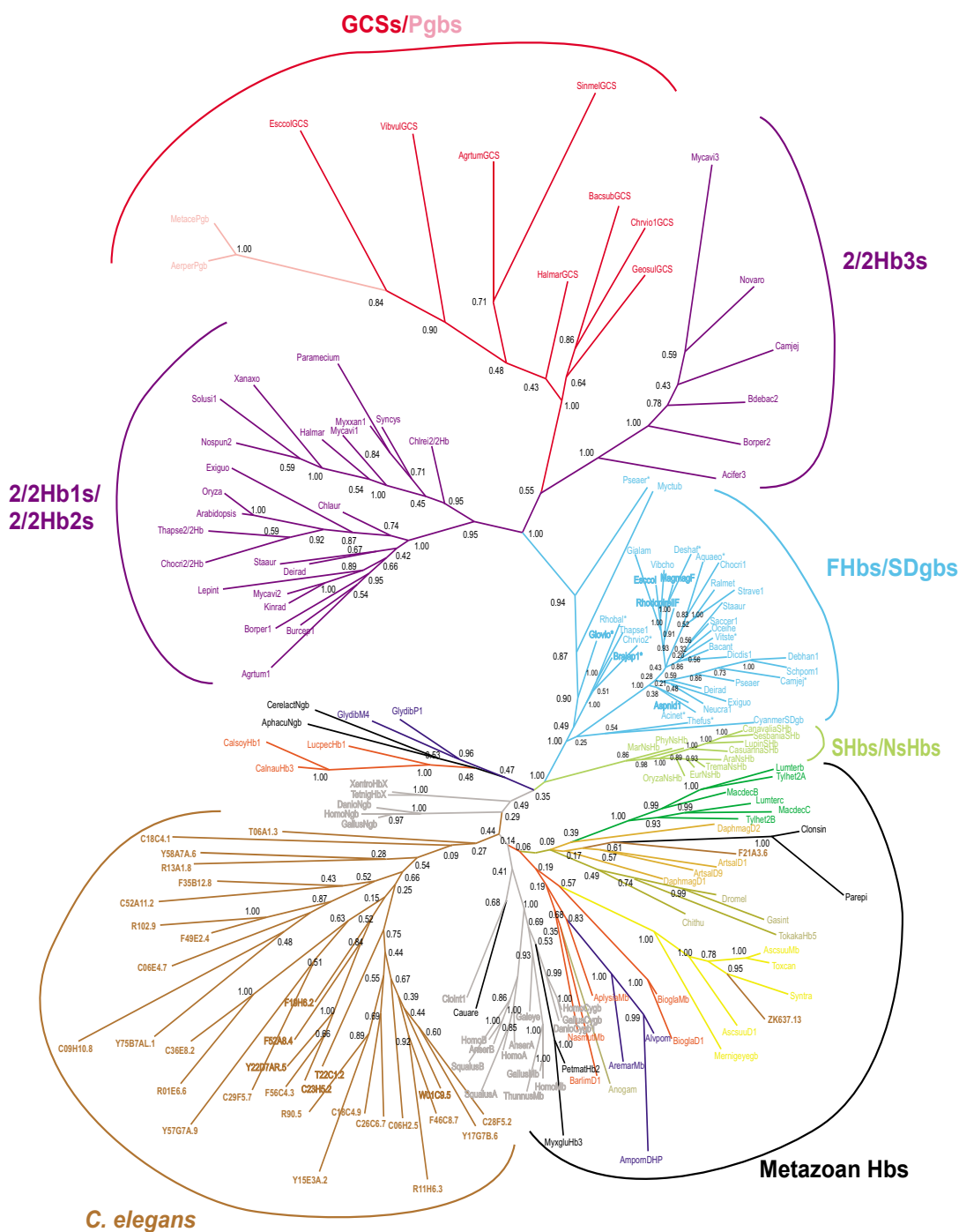
Since our census indicates that GCSs do not occur in eukaryotes, it is necessary to consider the heme-regulated eukaryotic initiation factor 2 α kinase (~630aa), which was found recently to have two heme-binding domains, of which the N-terminal domain was considered to be globin-like [89,90]. Although it was demonstrated that His78 and His123 were the two residues involved in heme binding [91], we find that the N-terminal 138aa of the rabbit protein (gi|462439|P33279|E2AK1 RABIT) is not recognized as a globin in CD or FUGUE searches. A blastp search of the GenBank database found at least 6 mammalian counterparts; clearly, a structure is required to determine whether these sequences are globins.

Molecular evolution

The broad distribution of the three lineages of globins, the 3/3 FHbs/SDgbs, the 3/3 GCS/Pgbs and the 2/2 Hbs, is illustrated in the Bayesian phylogenetic tree shown in Fig. 5, based on a global manual alignment of 175 sequences representing all the major groups of globins (Supplemental Data Fig. 3, [see Additional File 2]). The 2/2 Hbs and the GCSs/Pgbs cluster separately (upper left hand side), with the three classes of 2/2 Hbs clearly delineated. Within the 2/2Hb clusters, the archaeal (*Haloarcula*), ciliate (*Paramecium*) and chlorophyte (*Chlamydomonas*) globins occur with the class 1 and the diatom (*Thalassiosira*) and plant (*Oryza*, *Arabidopsis*) Hbs group with the class 2. The remaining stem encompasses the bacterial and eukaryotic FHbs and related SDgbs (marked with an aster-

isk), including the *Cyanidioschyzon* and *Thalassiosira* SDgbs, the separate clusters of plant NsHbs and SHbs (lower right hand side) and all the known metazoan globins, including the vertebrate globins (lower left hand side). Noteworthy is the clustering of bacterial SDgbs with the globin domains of bacterial and eukaryote (fungal, diplomonad and mycetozoa) FHbs, suggestive of past lateral gene transfer events [49]. Likewise, the clustering of the archaeal 2/2 Hbs with the class 1 bacterial 2/2 Hbs, could be indicative of a past lateral gene transfer. Very recently, a 2/2Hb and an FHb were found in the multicellular red alga *Chondrus crispus* (X. Bailly *et al.*, unpublished results); these two sequences cluster with the *Thalassiosira* 2/2Hb and *Aquifex* SDgb, respectively. An interesting result is the close grouping of *Amphitrite ornata* dehaloperoxidase [92] with the intracellular hemoglobin of the hydrothermal vent annelid *Alvinella pompejana* [93], suggesting that the latter may be a dehaloperoxidase also. The *Gallus* and *Xenopus* HbX group with the vertebrate Ngbs as found earlier [40]. Of particular interest is the large *C. elegans* globin family: all except F21A3.6 and ZK637.13, cluster together, and appear to be closest to the vertebrate Ngbs. ZK637.13 groups with globins from other nematode species, F21A3.6 clusters with crustacean (*Daphnia* and *Artemia*) and platyhelminth (*Paramphistomum* and *Clonorchis*) sequences. It should be noted that the locations of these three globins is dependent on the number of globin sequences used to construct the phylogenetic tree.

We have discussed elsewhere [49] the possible evolutionary scenarios for the emergence of the proposed three lineages of globins in two structural classes: (1) the 3-over-3 SD globins and FHbs, (2) the 3-over-3 GCS/Pgbs, and (3), the 2-over-2 SDgbs. Here, we would like to reiterate our proposal that all metazoan and plant globins originated from a SDgb related to the present day bacterial and algal SDgbs and the N-terminal globin domains of fungal and bacterial FHbs. This proposal is based on two results, one of which, is the clustering of all metazoan and plant sequences with the bacterial and eukaryote FHbs and SDgbs and separate from the branches encompassing 2/2 Hbs and GCSs/Pgbs, in the Bayesian phylogenetic tree obtained earlier [49] and in the one shown in Fig. 5. Another key result, is that bacterial SDgbs and eukaryote FHb globin domains used as queries in iterated psiblast searches, recognize (i.e. have E values substantially lower than threshold) vertebrate globins, particularly Ngbs, and other metazoan globin groups, ahead of the 2/2 Hbs and GCSs/Pgbs [49]. Conversely, vertebrate Ngbs as a group, recognize the bacterial SDgbs ahead of other vertebrate globins. Furthermore, the tree shown in Fig. 5 is in very good agreement with recent models of vertebrate globin evolution [36,62,94], wherein duplication of the ancestral globin gene resulted in neuroglobin and cellular globin

**Figure 5**

A Bayesian phylogenetic tree based on 175 sequences representing all the known globin families (alignment provided in Supplemental Data Fig. 3). The scale bar represents a distance of 0.1 accepted amino acid mutations per site. The numbers at the nodes represent Bayesian posterior probabilities.

genes, with subsequent duplication of the latter into cellular and Hb gene loci, followed by additional duplications of the cellular globin locus into Mb and Cygb and of the Hb gene into the α - and β -globin genes.

The first attempt at defining the molecular phylogeny of globins [95] was a tree based on 245 sequences, all from eukaryotes. A subsequent study by Moens *et al.* [96] based on 700 sequences, including bacterial ones, proposed that all globins «evolved from a family of ancestral, ca. 17 kDa hemeproteins, which displayed the globin fold and functioned as redox proteins». The present survey of extant globins in the three kingdoms of life provides some interesting additional details: we now know that there are three lineages of globins, two with a 3/3 α -helical fold and one with the 2/2 fold, and that only the latter occurs at present in all three kingdoms of life. Furthermore, it appears likely that formation of chimeric proteins containing globin domains occurred, as separate events, prior to the emergence of the three kingdoms of life. There are no obvious explanations for the absence of FHbs in Archaea and of GCSs in eukaryotes. Although it is not possible at present to decide which of the two folds originated from the other, the occurrence of 2/2 Hbs in all three kingdoms of life would suggest that it is the ancestral fold. A recent computational study of plant Hb folding showed that one of the folding modules of rice 3/3 NsHb overlaps the 2/2 fold of *Mycobacterium tuberculosis* HbO (class 2) suggesting that it is an ancient structural feature of globins [97].

Globin function

The presence of multiple globins in all three kingdoms of life raises the question of their function, particularly among the unicellular organisms. Although a complete discussion is not possible here, some general observations can be made, limited to organisms other than Animalia. The bacterial FHbs appear to have a role primarily in the detoxification of NO, via two NADH dependent activities, an NO dioxygenase activity under aerobic conditions, or an NO reductase activity under anaerobic conditions [42,43,98-100]. Furthermore, the recent demonstration that the *E. coli* FHb binds lipids and is an efficient alkylhydroperoxide reductase, suggests that it may be involved in the repair of lipid membranes damaged by oxidative/nitrosative stress [101-103]. The function(s) of bacterial SDgbs appear to be somewhat similar to the FHbs. Although the function of *Vitreoscilla* Hb is to increase the effective intracellular O₂ concentration under microaerobic conditions [42,104], that of *Campylobacter* SDgb was shown recently to function only in NO scavenging and detoxification and not provide resistance against superoxide or peroxides [105]. In eukaryotes, such as yeasts and *Dictyostelium*, FHbs also provide protection against NO [43,106,78], as well as enhancement of respiration, directly by functioning as an O₂ buffer and indirectly, by

reducing the NO concentration in the mitochondria which otherwise inhibits respiration [107-110]. In pathogenic microorganisms, FHb provides protection from human macrophage NO-mediated killing and promotes the virulence of bacteria, e.g. *Salmonella* [111], and of yeasts, *Candida* [112] and *Cryptococcus neoformans*, a worldwide pathogen causing pulmonary infection in animals and humans [113,114]. It is interesting to note, as suggested by one of the reviewers, that since most of the Archaea are not pathogenic, they may have dispensed with the FHbs and their protective role in bacteria.

In plants, the SHbs have been shown to be required for establishing a low oxygen concentration for the effective functioning of the bacterial nitrogenase necessary for nitrogen fixation [115]. Although the role of 2/2 Hbs in plants is completely unknown, the NsHbs, induced in plant cells upon exposure to low oxygen concentrations, are thought to play a role in a metabolic pathway which also involves nitric oxide and which provides an alternative type of respiration to the mitochondrial electron transport under hypoxic conditions [116,117].

The GCSs represent one of the four known families of heme-based sensors [118], and can be subdivided into two groups, the HemATs and the gene regulators. The former are aerotactic heme sensors, with a C-terminal domain that is related to chemotaxis methyl-accepting proteins. Although *Bacillus subtilis* HemAT elicits an aerophilic response, that from the archaean *Halobacterium salinarum* provides an aerophobic response [44,45]. The gene regulators have C-terminal domains, some of which may regulate second messengers and others that have unknown functions [47,48,59,118]. Although little is known about the specific function of the Pgbs, they have a Cys at position E19, similar to globins of the annelids living in sulfide-rich environments, including deep sea hydrothermal vents and marine sediments, where this residue has been implicated in sulfide binding [119,120].

Recent studies of *Mycobacterium tuberculosis* 2/2 Hbs, HbN (2/2 Hb1) and HbO (2/2Hb2), indicate that the former functions in NO detoxification, while HbO, which differs substantially from HbN in structure [121], is expressed in association with cell membranes and significantly enhances respiration, suggesting an interaction with the electron transport chain [122-126]. It remains to be seen whether these findings apply to other bacterial 2/2 Hbs. Although nothing is known about the function of the 2/2Hb3s, it is safe to assume that it must differ from the other two classes, since 32 bacteria have two and *Mycobacterium avium* ssp. *tuberculosis* and *Methylococcus capsulatus* have all three classes of 2/2 Hbs.

Finally, it is appropriate to mention here the case of the extracellular Hb from the nematode *Ascaris suum*, an octamer of two-domain globin chains, long known for its high oxygen affinity [127], whose function appears to be that of an NO reductase [128], similar to that of fungal and bacterial FHbs. Interestingly FUGUE searches using FHb and SDgb sequences as queries, invariably include the *Ascaris* Hb domain 1 structure (1ash) among the highest scoring globin structures. This case provides an additional bit of evidence in support of our proposal that the FHbs and related SDgbs from bacteria, algae and unicellular eukaryotes, including fungi, are part of one of the three globin lineages, and the one from which originated all metazoan globins and all plant SHbs and NsHbs [49].

Conclusion

The phylogenomic profile derived from our survey of genomes from the three kingdoms of life presented here, delineates the present day limits of the occurrence of the three lineages of globins, and provides a clear view of the work that remains to be done. It appears likely that in contrast to archaea, where ~20% of the known genomes have globins, the majority of bacteria will be shown to have globins. Based on the prevailing opinion that all plants have globins, it is likely that this will also hold true for the unicellular, photosynthesizing eukaryotes. Globin occurrence in other unicellular eukaryotes is likely to be episodic, just as in the case of nonvertebrate metazoans.

Another obvious conclusion is that globins are mostly enzymes and less frequently sensors, and that transport of oxygen is a function that developed relatively recently, accompanying the emergence of multicellular organisms. There are at least two more known instances of evolution from enzyme to transporter. One, is the convergent evolution of indoleamine dioxygenase into a muscle heme protein with Mb-like oxygen binding properties, in gastropod molluscs [129]. Another are hemocyanins, the copper containing respiratory proteins in molluscs and arthropods, which have evolved from phenoloxidases, prior to the divergence of Protostomes and Deuterostomes [130]. The recent finding of hemerythrins, similar to the non-heme iron respiratory protein of Sipunculids, Brachiopods and Priapulids, in the methanotrophic Gammaproteobacterium *Methylococcus capsulatus*, and in other prokaryotes [131], and also in the hydrothermal vent annelid *Riftia pachyptila* (X. Bailly et al., unpublished observations), where they may have an enzymatic function, suggests yet another possible instance.

Methods

Identification of globin sequences

Putative globins and globin domains were identified in the genomes of 49 eukaryotes, 26 archaea and 245 bacteria, listed in Supplementary Data Table 1, using two

approaches. In one, we examined the gene assignments based on a library of hidden Markov models [132], listed on the SUPERFAMILY site <http://supfam.mrc-lmb.cam.ac.uk>, discarded sequences shorter than 100aa and checked the alignments for the presence of His at Mb-fold position F8. In the other, we performed blastp and tblastn (version 9.2.2) searches with pairwise alignment [133], of completed and unfinished genomes in the GenBank, using the NCBI Entrez retrieval system <http://www.ncbi.nlm.nih.gov/BLAST/>, including the genomes of the rhodophyte (red alga) *Cyanidioschyzon merolae* <http://merolae.biol.s.u-tokyo.ac.jp>, chlorophyte (green algae) *Chlamydomonas reinhardtii* <http://www.biology.duke.edu/chlamy/>, and the diatoms *Phaeodactylum tricornutum* <http://avesthagen.szbowler.com> and *Thalassiosira pseudonana* <http://genome.jgi-psf.org/thaps1/thaps1.home.html>.

Blastp searches use the Expect value (E) to assess the matches between the query sequence and each of the sequences in a database. Thus, E = 0.1 signifies that the probability of finding by chance, another match with the query sequence having the same score, is 1 in 10. We define recognition to be a hit with E < 0.005, the default threshold, and with the pairwise alignment fulfilling the following two criteria: proper alignment of the F8 His residues and of helices BC through G. It should be noted that blastp searches often misalign the E helices when the E7 residues are different, e.g. His and Q; however, the rest of the alignment is unaffected.

In cases where the identification of a putative globin was uncertain, searches employing CD-Search v.2.02 [134] <http://www.ncbi.nlm.nih.gov>, PFAM [135] <http://www.sanger.ac.uk> and FUGUE [136] <http://www-cryst.bioc.cam.ac.uk> were used to determine whether the borderline sequence should be accepted as a globin.

Alignment of sequences

The putative globin sequences were aligned manually, using the procedure employed earlier in the alignment of over 700 globins [137], based on the myoglobin fold [138,139], the pattern of predominantly hydrophobic residues at 37 conserved, solvent-inaccessible positions with mean solvent-accessible areas of <15Å² [140], including 33 intra-helical residues defining helices A through H, A8, A11, A12, A15, B6, B9, B10, B13, B14, C4, E4, E7, E8, E11, E12, E15, E18, E19, F1, F4, G5, G8, G11, G12, G13, G15, G16, H7, H8, H11, H12, H15, and H19, the three inter-helical residues at CD1, CD4 and FG4, and the invariant His at F8. Only amino acids which occur at the 33 intra-helical positions in the foregoing alignment were allowed in the alignment of the putative globin sequences. Although earlier alignments by Kapp et al. [137] and by Moens et al. [96] had indicated that there were two invariant residues in globins, F8His and CD1Phe, the 2/2Hb family can accommodate other hydrophobic residues,

such as Tyr/Met/Leu/Ile/Val at the CD1 position as well as Ala/Ser/Thr/Leu at the distal E7 position, in addition to His and Gln [16,20-22]. Hence, in our alignments, we required a His at the proximal F8 position, a residue at the distal E7 position in the order of preference His>Gln>Leu>Thr>Ala>Val>Ser>Tyr, a hydrophobic residue at position CD1 in the order of preference Phe>Tyr>Leu>Met>Ile>Val. At position C4, usually a Pro, we accepted Ala or Ser or Thr but not a charged residue. At the interhelical position CD4, we sought a hydrophobic residue, when available, and at position FG4 we placed a hydrophobic residue in the order of preference Ile>Leu>Val>Met>Phe>Tyr. Furthermore, we avoided deletions in any of the helical regions and placed no limit on the number of residues within the interhelical regions.

Molecular phylogeny

Bayesian phylogenetic trees were obtained employing MrBayes Version 3.1.1 [141]; four chains were run simultaneously for 2000000 generations and trees were sampled every 100 generations generating a total of 20000 trees. PAUP version 4.0b10 [142] was used for viewing and editing. The JTT transition matrix [143] was used as the stochastic model of amino acid substitution.

Abbreviations

Cygb – 3-over-3 cytoglobin; FHb – flavohemoglobin, chimeric proteins (~400aa) comprising a 3-over-3 N-terminal globin and a C-terminal flavin reductase domain; GCS – globin-coupled sensors: chimeric proteins (~300 to >700aa) comprising a 3-over-3 N-terminal globin domain and a variable C-terminal portion; Hb – hemoglobin; SHb – 3-over-3 symbiotic Hbs of legumes and other plants; Mb – myoglobin; Ngb – 3-over-3 neuroglobin; NsHbs – 3-over-3 nonsymbiotic plant Hbs; Pgb – protoglobin, single domain 3-over-3 globin related to the N-terminal domain of GCSs; SDgb – 3-over-3 single domain globin (~140aa) related to the N-terminal of FHbs; 3/3gbs – all globins that have the canonical 3-over-3 α -helical fold; 2/2 Hbs – "truncated" Hbs that are not necessarily shorter than ~140aa, which have the 2-over-2 α -helical fold.

Authors' contributions

SNV identified globin sequences from genomic data and performed the alignments.

DH identified globin sequences and participated in sequence alignment and construction of phylogenetic trees.

XB identified globin sequences and participated in sequence alignment and construction of phylogenetic trees.

RAP provided systematic input and was involved in the preparation of the MS.

JG participated in the identification of globin sequences.

SD provided systematic input and was involved in the preparation of the MS.

LM provided systematic input and was involved in the preparation of the MS.

JVF provided systematic input and was involved in the preparation of the MS.

Additional material

Additional File 1

Table 1. List of genomes from the three kingdoms of life used in the present study. Table 2. Identified and putative globins in archaeal genomes. Table 3. Phylogenomic distribution of identified and putative globins in bacteria. Table 4. Identified and putative globins in eukaryote genomes. Table 5. The putative globin orthologs of *Caenorhabditis briggsae* and *C. elegans*. Table 6. Identified and putative globins in fungal genomes.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2148-6-31-S1.doc>]

Additional File 2

Fig. 1. Alignment of selected globin sequences. Fig. 2. Alignment of putative globins from *Caenorhabditis briggsae* and *C. elegans*. Fig. 3. Alignment of 175 representative globin sequences used in the construction of the Bayesian phylogenetic tree shown in Fig. 5.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2148-6-31-S2.doc>]

Acknowledgements

This work was supported by grants from the Fund for Scientific Research Flanders (G.0331.04), the European Commission (QLG3-CT-2002-01548) and the Consejo Nacional de Ciencia y Tecnología (project no. 42873-Q), México. SD is a postdoctoral fellow of the Fund for Scientific Research Flanders (FWO).

References

1. Appleby C: **Leghemoglobin and rhizobium respiration.** *Ann Rev Plant Physiol* 1984, **34**:443-478.
2. Vinogradov SN, Walz DA, Pohajdak B, Moens L, Kapp O, Suzuki T, Trotman C: **Adventitious variability? The amino acid sequences of nonvertebrate globins.** *Comp Biochem Physiol* 1993, **B106**:1-26.
3. Weber RE, Vinogradov SN: **Nonvertebrate hemoglobins: functions and molecular adaptations.** *Physiol Rev* 2001, **81**:569-628.
4. Arredondo-Peter R, Hargrove MS, Moran J, Sarath G, Klucas RV: **Plant hemoglobins.** *Plant Physiol* 1998, **118**:1121-1125.
5. Ross E, Lira-Ruan V, Arredondo-Peter R, Klucas R, Sarath G: **Recent insights into plant hemoglobins.** *Rev Plant Biochem Biotechnol* 2002, **1**:173-189.
6. Dordas C, Rivoal J, Hill RD: **Plant haemoglobins, nitric oxide and hypoxic stress.** *Ann Bot (Lond)* 2003, **91**:173-178.

7. Vasudevan S, Armarego W, Shaw D, Lilley P, Dixon N, Poole RK: **Isolation and nucleotide sequence of the hmp gene that encodes a haemoglobin-like protein in Escherichia coli K-12.** *Mol Gen Genet* 1991, **226**:49-58.
8. Iwaasa H, Takagi T, Shikama K: **Amino acid sequence of yeast hemoglobin. A twodomain structure.** *J Mol Biol* 1992, **227**:948-954.
9. Zhu H, Riggs A: **Yeast flavohemoglobin is an ancient protein related to globins and a reductase family.** *Proc Natl Acad Sci USA* 1992, **89**:5015-5019.
10. Takagi T: **Hemoglobins from single-celled organisms.** *Curr Opin Struct Biol* 1993, **3**:413-418.
11. Potts M, Angeloni S, Ebel R, Bassam D: **Myoglobin in a cyanobacterium.** *Science* 1992, **256**:1690-1692.
12. Kaneko T, Tabata S: **Complete genome structure of the unicellular cyanobacterium Synechocystis sp. PCC6803.** *Plant Cell Physiol* 1997, **38**:1171-1176.
13. Scott N, Falzone C, Vuletich D, Zhao J, Bryant V, Lecomte JT: **Truncated hemoglobin from the cyanobacterium Synechococcus sp. PCC 7002: evidence for hexacoordination and covalent adduct formation in the ferric recombinant protein.** *Biochemistry* 2002, **41**:6902-6910.
14. Vandergon T, Riggs C, Gorr T, Colacino J, Riggs A: **The mini-hemoglobins in neural and body wall tissue of the nemertean worm Cerebratulus lacteus.** *J Biol Chem* 1998, **273**:16998-17011.
15. Couture M, Yeh S, Wittenberg BA, Wittenberg JB, Ouellet Y, Rousseau D, Guertin M: **A cooperative oxygen-binding hemoglobin from Mycobacterium tuberculosis.** *Proc Natl Acad Sci USA* 1999, **96**:1223-1228.
16. Wittenberg JB, Bolognesi M, Wittenberg BA, Guertin M: **Truncated hemoglobins: a new family of hemoglobins widely distributed in bacteria, unicellular eukaryotes, and plants.** *J Biol Chem* 2002, **277**:871-874.
17. Couture M, Chamberland H, St-Pierre B, Lafontaine J, Guertin M: **Nuclear genes encoding chloroplast hemoglobins in the unicellular green alga Chlamydomonas eugametos.** *Mol Gen Genet* 1994, **243**:185-197.
18. Couture M, Das T, Lee H, Peisach J, Rousseau D, Wittenberg BA, Wittenberg JB, Guertin M: **Chlamydomonas chloroplast ferrous hemoglobin. Heme pocket structure and reactions with ligands.** *J Biol Chem* 1999, **274**:6898-6910.
19. Watts R, Hunt P, Hvitved A, Hargrove M, Peacock W, Dennis E: **A hemoglobin from plants homologous to truncated hemoglobins of microorganisms.** *Proc Natl Acad Sci USA* 2001, **98**:10119-10124.
20. Pesce A, Couture M, Dewilde S, Guertin M, Yamauchi K, Ascenzi P, Moens L, Bolognesi M: **A novel two-over-two alpha-helical sandwich fold is characteristic of the truncated hemoglobin family.** *EMBO J* 2000, **19**:2424-2434.
21. Milani M, Pesce A, Ouellet Y, Ascenzi P, Guertin M, Bolognesi M: **Mycobacterium tuberculosis haemoglobin N displays a protein tunnel suited for O₂ diffusion to the haem.** *EMBO J* 2001, **20**:3902-3909.
22. Milani M, Savard P, Ouellet H, Ascenzi P, Guertin M, Bolognesi M: **A TyrCD1/TrpG8 hydrogen bond network and a TyrB10-TyrCD1 covalent link shape the heme distal site of Mycobacterium tuberculosis hemoglobin O.** *Proc Natl Acad Sci USA* 2003, **100**:5766-5771.
23. Blaxter ML: **Nemoglobins: divergent nematode globins.** *Parasitology Today* 1993, **9**:353-360.
24. Kloek A, Sherman D, Goldberg DE: **Novel gene structure and evolutionary context of Caenorhabditis elegans globin.** *Gene* 1993, **129**:215-221.
25. Kloek A, McCarter J, Setterquist R, Schedl T, Goldberg DE: **Caenorhabditis globin genes: rapid intronic divergence contrasts with conservation of silent exonic sites.** *J Mol Evol* 1996, **43**:101-108.
26. Blaxter ML, Ingram L, Tweedie S: **Sequence, expression and evolution of the globins of the parasitic nematode Nippostrongylus brasiliensis.** *Mol Biochem Parasitol* 1994, **68**:1-14.
27. Mansell J, Timms K, Tate W, Moens L, Trotman CN: **Expression of a globin gene in Caenorhabditis elegans.** *Biochem Mol Biol Int* 1993, **30**:643-647.
28. Vanfleteren JR, Van de Peer Y, Blaxter M, Tweedie S, Trotman C, Lu L, Van Hauwaert M, Moens L: **Molecular genealogy of some nematode taxa based on cytochrome c and globin amino acid sequences.** *Mol Phylogenet Evol* 1994, **3**:92-101.
29. Neuwald A, Liu J, Lipman D, Lawrence C: **Extracting protein alignment models from the sequence database.** *Nucl Acids Res* 1997, **25**:1665-1677.
30. Hoogewijs D, Geuens E, Dewilde S, Moens L, Vierstraete A, Vinogradov SN, Vanfleteren JR: **Genome-wide analysis of the globin gene family of C. elegans.** *IUBMB Life* 2004, **56**:697-702.
31. Burmester T, Hankeln T: **A globin gene of Drosophila melanogaster.** *Mol Biol Evol* 1999, **16**:1809-1811.
32. Ebner B, Burmester T, Hankeln T: **Globin genes are present in Ciona intestinalis.** *Mol Biol Evol* 2003, **20**:1521-1525.
33. Burmester T, Weich B, Reinhardt S, Hankeln T: **A vertebrate globin expressed in the brain.** *Nature* 2000, **407**:520-523.
34. Trent J, Watts R, Hargrove M: **Human neuroglobin, a hexacoordinate hemoglobin the reversibly binds oxygen.** *J Biol Chem* 2001, **276**:30106-30110.
35. Kawada N, Kristensen D, Asahina K, Nakatani K, Minamiyama Y, Seki S, Yoshizato K: **Characterization of a stellate cell-activation associated protein (STAP) with peroxidase activity found in rat hepatic stellate cells.** *J Biol Chem* 2001, **276**:25318-25323.
36. Burmester T, Ebner B, Weich B, Hankeln T: **Cytoglobin: a novel globin type ubiquitously expressed in vertebrate tissues.** *Mol Biol Evol* 2002, **19**:416-421.
37. Trent J, Hargrove MS: **A ubiquitously expressed human hexacoordinate hemoglobin.** *J Biol Chem* 2002, **277**:19538-19545.
38. Hankeln T, Ebner B, Fuchs C, Gerlach F, Haberkamp M, Laufs TL, Roesner A, Schmidt M, Weich B, Wystub S, Saaler-Reinhardt S, Reuss S, Bolognesi M, De Sanctis D, Marden MC, Kiger L, Moens L, Dewilde S, Nevo E, Avivi A, Weber RE, Fago A, Burmester T: **Neuroglobin and cytoglobin in search of their role in the vertebrate globin family.** *J Inorg Biochem* 2005, **99**:110-119.
39. Kugelstadt D, Haberkamp M, Hankeln T, Burmester T: **Neuroglobin, cytoglobin, and a novel, eye-specific globin from chicken.** *Biochem Biophys Res Commun* 2004, **325**:719-725.
40. Roesner A, Fuchs C, Hankeln T, Burmester T: **A globin gene of ancient evolutionary origin in lower vertebrates: evidence for two distinct globin families in animals.** *Mol Biol Evol* 2005, **22**:12-20.
41. Poole RK, Hughes MN: **New functions for the ancient globin family: bacterial responses to nitric oxide and nitrosative stress.** *Mol Microbiol* 2000, **36**:775-783.
42. Frey AD, Kallio PT: **Bacterial hemoglobins and flavohemoglobins: versatile proteins and their impact on microbiology and biotechnology.** *FEMS Microbiol Rev* 2003, **27**:525-545.
43. Wu G, Wainwright L, Poole RK: **Microbial globins.** *Adv Microb Physiol* 2003, **47**:255-310.
44. Hou S, Larsen R, Boudko D, Riley C, Karatan E, Zimmer M, Ordal G, Alam M: **Myoglobin-like aerotaxis transducers in Archaea and Bacteria.** *Nature* 2000, **403**:540-544.
45. Yu H, Saw J, Hou S, Larsen R, Watts K, Johnson M, Zimmer M, Ordal G, Taylor B, Alam M: **Aerotactic responses in bacteria to photoreleased oxygen.** *FEMS Microbiol Lett* 2002, **217**:237-242.
46. Hou S, Freitas T, Larsen R, Piatibratov M, Sivozhelzev V, Yamamoto A, Meleshkevitch E, Zimmer M, Ordal G, Alam M: **Globin-coupled sensors: a class of heme-containing sensors in Archaea and Bacteria.** *Proc Natl Acad Sci USA* 2001, **98**:9353-9358.
47. Freitas T, Hou S, Alam M: **The diversity of globin-coupled sensors.** *FEBS Lett* 2003, **552**:99-104.
48. Freitas T, Hou S, Dioum E, Saito J, Newhouse J, Gonzalez G, Gilles-Gonzalez MA, Alam M: **Ancestral hemoglobins in Archaea.** *Proc Natl Acad Sci USA* 2004, **101**:6675-6680.
49. Vinogradov SN, Hoogewijs D, Bailly X, Arredondo-Peter R, Guertin M, Gough J, Dewilde S, Moens L, Vanfleteren JR: **Three globin lineages belonging to two structural classes in genomes from the three kingdoms of life.** *Proc Natl Acad Sci USA* 2005, **102**:11385-11389.
50. Baldauf SL, Battacharya D, Cockrill J, Hugenholtz D, Pawlowski J, Simpson A: **The tree of life. An overview.** In *Assembling the Tree of Life* Edited by: Cracraft J, Donoghue MJ. Oxford University Press, Oxford, UK; 2004:43-75.
51. Zhang W, Phillips GN Jr: **Structure of the oxygen sensor in Bacillus subtilis. signal transduction of chemotaxis by control of symmetry.** *Structure* 2003, **11**:1097-1108.
52. Schleper C, Jurgens G, Jonuscheit M: **Genomic studies of uncultivated Archaea.** *Nature Rev Microbiol* 2005, **3**:479-488.

53. Vuletich D, Lecomte JTJ: **A phylogenetic and structural analysis of truncated hemoglobins.** *J Mol Evol* 2006, **62**:196-210.
54. Lira-Ruan V, Sarath G, Klucas RV, Arredondo-Peter R: **In silico analysis of a flavohemoglobin from *Sinorhizobium meliloti* strain 1021.** *Microbiol Res* 2003, **158**:215-227.
55. Wakabayashi S, Matsubara H, Webster D: **Primary sequence of a dimeric bacterial haemoglobin from *Vitreoscilla*.** *Nature* 1977, **266**:481-483.
56. Kim Y, Joachimiak A, Skarina T, Bochkarev A, Savchenko A, Edwards A: **Crystal structure of Pa3967 from *Pseudomonas aeruginosa* Paol, a hypothetical protein which is highly homologous to human hemoglobin in structure.** 2004. unpublished
57. Tarricone C, Galizzi A, Coda A, Ascenzi P, Bolognesi M: **Unusual structure of the oxygen-binding site in the dimeric bacterial hemoglobin from *Vitreoscilla* sp.** *Structure* 1997, **5**:497-507.
58. Ilari A, Bonamore A, Farina A, Johnson K, Boffi A: **The X-ray structure of ferric *Escherichia coli* flavohemoglobin reveals an unexpected geometry of the distal heme pocket.** *J Biol Chem* 2002, **277**:23725-23732.
59. Ermler U, Siddiqui R, Cramm R, Friedrich B: **Crystal structure of the flavohemoglobin from *Alcaligenes eutrophus* at 1.75 Å resolution.** *EMBO J* 1995, **14**:6067-6077.
60. Vandamme P, Coenye T: **Taxonomy of the genus *Cupriavidus*: a tale of lost and found.** *Int J Syst Evol Microbiol* 2004, **54**:2285-2289.
61. Freitas T, Saito J, Hou S, Alam M: **Globin-coupled sensors, protoglobins, and the last universal common ancestor.** *J Inorg Biochem* 2005, **99**:23-33.
62. Zhang W, Phillips GN Jr: **Structure of the oxygen sensor in *Bacillus subtilis*. signal transduction of chemotaxis by control of symmetry.** *Structure* 2003, **11**:1097-1108.
63. Moran NA: **Tracing the evolution of gene loss in obligate bacterial symbionts.** *Curr Opin Microbiol* 2003, **6**:512-518.
64. Goh S-H, Lee Y, Bhanu N, Cam M, Desper R, Martin B, Moharram R, Gherman R, Miller JL: **A newly discovered human α -globin gene.** *Blood* 2005, **106**:1466-1472.
65. Brownlie A, Hersey C, Oates A, Paw B, Falick B, Witkowska H, Flint J, Higgs D, Jessen J, Bahary N, Zhu H, Lin S, Zon L: **Characterization of embryonic globin genes of the zebrafish.** *Dev Biol* 2003, **255**:48-61.
66. Gillemans N, McMorow T, Tewari R, Wai A, Burgtorf C, Drabek D, Ventres M, Langeveld A, Higgs D, Tan-UN K, Grosveld F, Philipsen S: **Functional and comparative analysis of globin loci in pufferfish and humans.** *Blood* 2003, **101**:2842-2849.
67. Dehal P, Satou Y, Campbell RK, Chapman J, Degnan B, De Tomaso A, Davidson B, Di Gregorio A, Gelpke M, Goodstein DM, Harafuji N, Hastings KE, Ho I, Hotta K, Huang W, Kawashima T, Lemaire P, Martinez D, Meinertzhagen IA, Nuclea S, Nonaka M, Putnam N, Rash S, Saiga H, Satake M, Terry A, Yamada L, Wang HG, Awazu S, Azumi K, Boore J, Branno M, Chin-Bow S, DeSantis R, Doyle S, Francino P, Keys DN, Haga S, Hayashi H, Hino K, Imai KS, Inaba K, Kano S, Kobayashi K, Kobayashi M, Lee BL, Makabe KW, Manohar C, Matassi G, Medina M, Mochizuki Y, Mount S, Morishita T, Miura S, Nakayama A, Nishizaka S, Nomoto H, Ohta F, Oishi K, Rigoutsos I, Sano M, Sasaki A, Sasakura Y, Shoguchi E, Shin-i T, Spagnuolo A, Stainier D, Suzuki MM, Tassy O, Takatori N, Tokutomi M, Yagi K, Yoshizaki F, Wada S, Zhang C, Hyatt PD, Larimer F, Detter C, Doggett N, Glavina T, Hawkins T, Richardson P, Lucas S, Kohara Y, Levine M, Satoh N, Rokhsar DS: **The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins.** *Science* 2002, **298**:2157-2167.
68. Small D, Moylan T, Vayda M, Sidell BD: **The myoglobin gene of the Antarctic icefish, *Chaenocephalus aceratus*, contains a duplicated TATAAAA sequence that interferes with transcription.** *J Exp Biol* 2006, **131**:139.
69. Rubin GM, Yandell MD, Wortman JR, Gabor Miklos GL, Nelson CR, Hariharan IK, Fortini ME, Li PW, Apweiler R, Fleischmann W, Cherry JM, Henikoff S, Skupski MP, Misra S, Ashburner M, Birney E, Boguski MS, Brody T, Brokstein P, Celniker SE, Chervitz SA, Coates D, Cravchik A, Gabriellian A, Galle RF, Gelbart WM, George RA, Goldstein LS, Gong F, Guan P, Harris NL, Hay BA, Hoskins RA, Li J, Li Z, Hynes RO, Jones SJ, Kuehl PM, Lemaitre B, Littleton JT, Morrison DK, Mungall C, O'Farrell PH, Pickeral OK, Shue C, Vossell LB, Zhang J, Zhao Q, Zheng XH, Lewis S: **Comparative genomics of the eukaryotes.** *Science* 2002, **297**:2204-2215.
70. Hankeln T, Jaenicke V, Kiger L, Dewilde S, Ungerechts G, Schmidt M, Urban J, Marden M, Moens L, Burmester T: **Characterization of *Drosophila* hemoglobin. Evidence for hemoglobin-mediated respiration in insects.** *J Biol Chem* 2002, **277**:29012-29017.
71. Richards S, Liu Y, Bettencourt B, Hradecky P, et al.: **Comparative genome sequencing of *Drosophila pseudoobscura*: chromosomal, gene and cis-element evolution.** *Genome Res* 2005, **15**:1-18.
72. Trevasakis B, Watts R, Andersson C, Llewellyn D, Hargrove MS, Olson JS, Dennis ES, Peacock WJ: **Two hemoglobin genes in *Arabidopsis thaliana*: the evolutionary origins of leghemoglobins.** *Proc Natl Acad Sci USA* 1997, **94**:12230-12234.
73. Arredondo-Peter R, Hargrove M, Sarath G, Moran J, Lohrman J, Olson JS, Klucas RV: **Rice haemoglobins. Gene cloning, analysis, and O-binding kinetics of a recombinant protein synthesized in *Escherichia coli*.** *Plant Physiol* 1997, **115**:1259-1266.
74. Lira-Ruan V, Ross E, Sarath G, Klucas RV, Arredondo-Peter R: **Mapping and analysis of a hemoglobin gene family from *Oryza sativa*.** *Plant Physiol Biochem* 2002, **40**:199-202.
75. Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, et al.: **Genome evolution in yeasts.** *Nature* 2004, **430**:35-44.
76. Sartori G, Aldegheri L, Mazzotta G, Lanfranchi G, Tournu H, Brown A, Carignani G: **Characterization of a new hemoprotein in the yeast *Saccharomyces cerevisiae*.** *J Biol Chem* 1999, **274**:5032-5037.
77. Andersson J, Sjogren A, Davis L, Embley T, Roger RJ: **Phylogenetic analyses of diplomonad genes reveal frequent lateral gene transfers affecting eukaryotes.** *Curr Biol* 2003, **13**:94-102.
78. Iijima M, Shimizu H, Tanaka Y, Urushihara H: **Identification and characterization of two flavohemoglobin genes in *Dictyostelium discoideum*.** *Cell Struct Funct* 2000, **25**:47-55.
79. Hemmingsen EA: **Respiratory and cardiovascular adaptation in hemoglobin-free fish: resolved and unresolved problems.** In *Biology of Antarctic Fish* Edited by: di Prisco G, Maresca B, Tota B. Springer-Verlag, New York; 1991:191-203.
80. Vayda M, Small D, Yuan M, Costello L, Sidell B: **Conservation of the myoglobin gene among Antarctic notothenioid fishes.** *Mol Mar Biol Biotechnol* 1997, **6**:207-216.
81. diPrisco G, Cocca E, Parker S, Detrich H: **Tracking the evolutionary loss of hemoglobin expression by the white-blooded Antarctic icefishes.** *Gene* 2002, **295**:185-191.
82. Bargelloni L, Lecointre G: **Four years of notothenioid systematics: a molecular perspective.** In *Fishes of Antarctica* Edited by: di Prisco G, Pisano E, Clarke A. Springer-Verlag Italia, Milan, Italy; 1998:259-273.
83. Bishop J, Vandergon T, Green D, Doeller J, Kraus D: **A high-affinity hemoglobin is expressed in the notochord of amphioxus, *Branchiostoma californiense*.** *Biol Bull* 1998, **195**:255-259.
84. Baker S, Terwilliger NB: **Hemoglobin structure and function in the rat-tailed sea cucumber *Paracaudina chilensis*.** *Biol Bull* 1993, **185**:115-122.
85. Christensen A, Colacino J, Bonaventura C: **Functional and biochemical properties of the hemoglobins of the burrowing brittle star *Hemipholis elongata* Say (Echinodermata, Ophiuroidea).** *Biol Bull* 2003, **205**:54-65.
86. Nielsen E, Mound L: **Global diversity of insects: the problem of estimating numbers.** In *Nature and Human Society: The Quest for a Sustainable World* Edited by: Raven PH. National Academy Press, Washington, DC; 1997:213-222.
87. Moreira D, Lopez-Garcia P: **The molecular ecology of microbial eukaryotes unveils a hidden world.** *Trends Microbiol* 2002, **10**:31-38.
88. Falkowski P, Katz M, Knoll A, Quigg A, Raven J, Schofield O, Taylor F: **The evolution of modern eukaryotic phytoplankton.** *Science* 2004, **305**:354-360.
89. Uma S, Matts R, Guo Y, White S, Chen JJ: **The N-terminal region of the heme-regulated eIF2 α kinase is an autonomous heme binding domain.** *Eur J Biochem* 2000, **267**:498-506.
90. Raffie-Kolpin M, Chefalo P, Hussain Z, Hahn J, Uma S, Matts R, Chen JJ: **Two heme-binding domains of heme-regulated eukaryotic initiation factor-2 α kinase. N terminus and kinase insertion.** *J Biol Chem* 2000, **275**:5171-5178.
91. Inuzuka T, Yun B, Ishikawa H, Takahashi S, Hori H, Matts R, Ishimori K, Morishima I: **Identification of crucial histidines for heme binding in the N-terminal domain of the heme-regulated eIF2 α kinase.** *J Biol Chem* 2004, **279**:6778-6782.
92. LaCount M, Zhang E, Chen Y, Han K, Whitton M, Lincoln D, Woodin S, Lebeda L: **The crystal structure and amino acid sequence of**

- dehaloperoxidase from *Amphitrite ornata* indicate common ancestry with globins. *J Biol Chem* 2000, **275**:18712-18716.
93. Hourdez S, Lallier F, De Cian M, Green B, Weber R, Toulmond A: **Gas transfer system in *Alvinella pompejana*: functional properties of intracellular and extracellular hemoglobins.** *Physiol Biochem Zool* 2000, **73**:365-373.
 94. Hardison RC: **Organization, evolution and regulation of the globin genes.** In *Disorders of Hemoglobin* Edited by: Steinberg MH, Forget BG, Higgs DR, Nagel RL. Cambridge University Press, Cambridge, UK; 2001:95-116.
 95. Goodman M, Pedwaydon J, Czelusniak J, Suzuki T, Gotoh T, Moens L, Shishikura F, Walz DA, Vinogradov SN: **An evolutionary tree for invertebrate globin sequences.** *J Mol Evol* 1988, **27**:236-249.
 96. Moens L, Vanfleteren J, Van de Peer Y, Peeters K, Kapp O, Czelusniak J, Goodman M, Blaxter M, Vinogradov SN: **Globins in nonvertebrate species: dispersal by horizontal gene transfer and evolution of the structure-function relationships.** *Mol Biol Evol* 1996, **13**:324-333.
 97. Nakajima S, Alvarez-Salgado E, Kikuchi T, Arredondo-Peter R: **Prediction of folding pathway and kinetics among plant hemoglobins using an average distance map method.** *Proteins Struct Funct Bioinform* 2005, **61**:500-506.
 98. Farres J, Rechsteiner M, Herold S, Frey AD, Kallio PT: **Ligand binding properties of bacterial hemoglobins and flavohemoglobins.** *Biochemistry* 2005, **44**:4125-4134.
 99. Gardner PM: **Nitric oxide dioxygenase function and mechanism of flavohemoglobins, hemoglobins, myoglobins and their associated reductases.** *J Inorg Biochem* 2005, **99**:247-266.
 100. Poole RK: **Nitric oxide and nitrosative stress tolerance in bacteria.** *Biochem Soc Trans* 2005, **33**:176-180.
 101. Bonamore A, Gentili P, Ilari A, Schinina M, Boffi A: **Escherichia coli flavohemoglobin is an efficient alkylhydroperoxide reductase.** *J Biol Chem* 2003, **278**:22272-22277.
 102. Bonamore A, Farina A, Gattoni M, Schinina M, Bellelli A, Boffi A: **Interaction with membrane lipids and heme ligand binding properties of *Escherichia coli* flavohemoglobin.** *Biochemistry* 2003, **42**:5792-5801.
 103. D'Angelo P, Lucarelli D, della Longa S, Benfatto M, Hazemann J, Feis A, Smulevich G, Ilari A, Bonamore A, Boffi A: **Unusual heme iron-lipid acyl chain coordination in *Escherichia coli* flavohemoglobin.** *Biophys J* 2004, **86**:3882-3892.
 104. Frey AD, Kallio PT: **Nitric oxide detoxification – a new era for bacterial globins in biotechnology?** *Trends Biotechnol* 2005, **23**:69-73.
 105. Elvers K, Wu G, Gilberthorpe N, Poole RK, Park SF: **Role of an inducible single-domain hemoglobin in mediating resistance to nitric oxide and nitrosative stress in *Campylobacter jejuni* and *Campylobacter coli*.** *J Bacteriol* 2004, **186**:5332-5341.
 106. Wu G, Wainwright L, Membrillo-Hernández J, Poole RK: **Bacterial hemoglobins: old proteins with "new" functions? Roles of respiratory and nitric oxide metabolism.** In *Respiration in Archaea and Bacteria, Diversity of Prokaryotic Electron Transport Carriers Volume 1*. Edited by: Zannoni D. Academic Press, London, UK; 2004:255-310.
 107. Buisson N, Labbe-Bois R: **Flavohemoglobin expression and function in *Saccharomyces cerevisiae*. No relationship with respiration and complex response to oxidative stress.** *J Biol Chem* 1998, **273**:9527-9533.
 108. Elvers K, Turner S, Wainwright L, Marsden G, Hinds J, Cole J, Poole RK, Penn C, Park SF: **NssR, a member of the Crp-Fnr superfamily from *Campylobacter jejuni*, regulates a nitrosative stress-responsive regulon that includes both a single-domain and a truncated haemoglobin.** *Mol Microbiol* 2005, **57**:735-750.
 109. Liu L, Zeng M, Hausladen A, Heitman J, Stamler J: **Protection from nitrosative stress by yeast flavohemoglobin.** *Proc Natl Acad Sci USA* 2000, **97**:4672-4676.
 110. Shikama K, Matsuoka A: **Structure-function relationships in unusual nonvertebrate globins.** *Crit Rev Biochem Mol Biol* 2004, **39**:217-259.
 111. Stevanin T, Poole RK, Demoncheaux E, Read R: **Flavohemoglobin Hmp protects *Salmonella enterica* serovar typhimurium from nitric oxide-related killing by human macrophages.** *Infect Immun* 2000, **70**:4399-4405.
 112. Ullmann B, Myers H, Chirananand W, Lazzell A, Zhao Q, Vega L, Lopez-Ribot J, Gardner PR, Gustin M: **Inducible defense mechanism against nitric oxide in *Candida albicans*.** *Eukaryote Cell* 2004, **3**:715-723.
 113. de Jesus-Berrios M, Liu L, Nussbaum J, Cox G, Stamler J, Heitman J: **Enzymes that counteract nitrosative stress promote fungal virulence.** *Curr Biol* 2003, **13**:1963-1968.
 114. Idnurm A, Reedy J, Nussbaum J, Heitman J: ***Cryptococcus neoformans* virulence gene discovery through insertional mutagenesis.** *Eukaryote Cell* 2004, **3**:420-429.
 115. Ott T, van Dongen J, Gunther C, Krusell L, Desbrosses G, Vigeolas H, Bock V, Czechowski T, Geigenberger P, Udvardi MK: **Symbiotic leghemoglobins are crucial for nitrogen fixation in legume root nodules but not for general plant growth and development.** *Curr Biol* 2005, **15**:531-5.
 116. Kundu S, Trent J, Hargrove M: **Plants, humans and hemoglobins.** *Trends Plant Sci* 2003, **8**:387-393.
 117. Igamberdiev A, Baron K, Manac'h-Little N, Stoimenova M, Hill RD: **The haemoglobin/nitric oxide cycle: involvement in flooding stress and effects of hormone signaling.** *Ann Bot* 2005, **96**:557-564.
 118. Gilles-Gonzalez MA, Gonzalez G: **Heme-based sensors: defining characteristics, recent developments, and regulatory hypotheses.** *J Inorg Biochem* 2005, **99**:1-22.
 119. Bailly X, Jollivet D, Vanin S, Deutsch J, Zal F, Lallier F, Toulmond A: **Evolution of the sulfide-binding function within the globin multigenic family of the deep-sea hydrothermal vent tubeworm *Riftia pachyptila*.** *Mol Biol Evol* 2002, **19**:1421-1433.
 120. Bailly X, Leroy R, Carney S, Collin O, Zal F, Toulmond A, Jollivet D: **The loss of the hemoglobin H₂S-binding function in annelids from sulfide-free habitats reveals molecular adaptation driven by Darwinian positive selection.** *Proc Natl Acad Sci USA* 2003, **100**:5885-5890.
 121. Lecomte JTL, Vuletich D, Lesk AM: **Structural divergence and distant relationships in proteins: evolution of the globins.** *Curr Opin Struct Biol* 2005, **15**:290-301.
 122. Ouellet H, Ouellet Y, Richard C, Labarre M, Wittenberg BA, Wittenberg JB, Guertin M: **Truncated hemoglobin HbN protects *Mycobacterium bovis* from nitric oxide.** *Proc Natl Acad Sci USA* 2002, **99**:5902-5907.
 123. Pathania R, Navani N, Rajamohan G, Dikshit KL: ***Mycobacterium tuberculosis* hemoglobin HbO associates with membranes and stimulates cellular respiration of recombinant *Escherichia coli*.** *J Biol Chem* 2002, **277**:15293-15302.
 124. Pathania R, Navani N, Gardner AM, Gardner PR, Dikshit KL: **Nitric oxide scavenging and detoxification by the *Mycobacterium tuberculosis* haemoglobin.** *Mol Microbiol* 2002, **45**:1303-1314.
 125. Milani M, Pesce A, Ouellet H, Guertin M, Bolognesi M: **Truncated hemoglobins and nitric oxide action.** *IUBMB Life* 2003, **55**:623-627.
 126. Mukai M, Savard P, Ouellet H, Guertin M, Yeh S: **Unique ligand-protein interactions in a new truncated hemoglobin from *Mycobacterium tuberculosis*.** *Biochemistry* 2002, **41**:3897-3905.
 127. Goldberg DE: **Oxygen-avid hemoglobin of *Ascaris*.** *Chem Rev* 1999, **99**:3371-3378.
 128. Minning D, Gow A, Bonaventura J, Braun R, Dewhirst M, Goldberg D, Stamler J: ***Ascaris* haemoglobin is a nitric oxide-activated 'deoxygenase'.** *Nature* 1999, **401**:497-502.
 129. Suzuki T, Imai K: **Evolution of myoglobin.** *Cell Mol Life Sci* 1998, **54**:979-1004.
 130. Immesberger A, Burmester T: **Putative phenoloxidases in the tunicate *Ciona intestinalis* and the origin of the arthropod hemocyanin superfamily.** *J Comp Physiol* 2004, **B174**:169-180.
 131. Karlsen O, Ramsevik L, Bruseth L, Larsen Ø, Brenner A, Berven F, Jensen H, Lillhaug J: **Characterization of a prokaryotic haemerythrin from the methanotrophic bacterium *Methylococcus capsulatus* (Bath).** *FEBS J* 2005, **272**:2428-2440.
 132. Gough J, Karplus K, Hughey R, Chothia C: **Assignment of homology to genome sequences using a library of hidden Markov models that represent all proteins of known structure.** *J Mol Biol* 2001, **313**:903-919.
 133. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: **Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.** *Nucleic Acids Res* 1997, **25**:3389-3402.
 134. Marchler-Bauer A, Bryant S: **CD-Search: protein domain annotations on the fly.** *Nucleic Acids Res* 2004, **32**:W327-331.
 135. Bateman A, Birney E, Cerruti L, Durbin R, Ewinger L, Eddy S, Griffiths-Jones S, Howe K, Marshall M, Sonnhammer E: **The Pfam protein families database.** *Nucleic Acids Res* 2002, **30**:276-280.

136. Shi J, Blundell T, Mizuguchi K: **FUGUE: sequence-structure homology recognition using environment-specific substitution tables and structure-dependent gap penalties.** *J Mol Biol* 2001, **310**:243-257.
137. Kapp O, Moens L, Vanfleteren J, Trotman C, Suzuki T, Vinogradov SN: **Alignment of 700 globin sequences: extent of amino acid substitution and its correlation in volume.** *Protein Sci* 1995, **4**:2179-2190.
138. Lesk AM, Chothia C: **How different amino acid sequences determine similar protein structures. The structure and evolutionary dynamics of the globins.** *J Mol Biol* 1980, **136**:225-270.
139. Bashford D, Chothia C, Lesk AM: **Determinants of a protein fold. Unique features of the globin amino acid sequences.** *J Mol Biol* 1987, **196**:199-216.
140. Gerstein M, Sonnhammer ELL, Chothia C: **Volume changes in protein evolution.** *J Mol Biol* 1994, **236**:1067-1078.
141. Huelsenbeck JP, Ronquist F: **MRBAYES: Bayesian inference of phylogenetic trees.** *Bioinformatics* 2001, **17**:754-755.
142. Swofford D: **Phylogenetic Analysis Using Parsimony.** In *Ver.4.0b 10* Sinauer Associates; 2001.
143. Jones DT, Taylor WR, Thornton JM: **The rapid generation of mutation data matrices from protein sequences.** *Cabios* 1992, **8**:275-282.
144. Singleton P, Sainsbury D: **Dictionary of Microbiology and Molecular Biology.** 3rd edition. J. Wiley & Sons, Chichester, UK; 2001.
145. Singleton P: **Bacteria in Biology, Biotechnology and Medicine.** 6th edition. J. Wiley & Sons, Chichester, UK; 2004.
146. Jaillon O, Aury J-M, Brune F, et al.: **Genome duplication in the teleost fish Tetraodon nigroviridis reveal the early vertebrate proto-karyotype.** *Nature* 2004, **431**:946-975.
147. Kellis M, Patterson N, Endrizzi M, Birren B, Landers ES: **Sequencing and comparison of yeast species to identify genes and regulatory element.** *Nature* 2003, **423**:241-254.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

